DIET OF FIVE COMMON ANURANS FOUND IN DISTURBED AREAS IN NORTHERN PENINSULAR MALAYSIA

by

YAP CHEE HUI

A thesis submitted in fulfilment of the requirements for the degree of Master of Science in Zoology.

February 2015

ACKNOWLEDGEMENT

I would like to express my utmost gratitude to my supervisor Prof. Ibrahim Jaafar for his guidance, help and advice during the period of my study. His understanding and support not only brought about the fruition of this thesis, but also boost my personal development and character building.

Also, many thanks to the members of my supervisory committee, for their suggestions and constructive critiques. I am also grateful for the assistance and recommendations from my fellow colleagues: Amirah Hurzaid and Zalina Awang; and for the dedication and support of field assistant Mohd Azmeer Abu Bakar. I am grateful to my family, for their unwavering support and encouragement.

Additionally, I would like to thank the School of Biological Sciences and School of Distance Education, Universiti Sains Malaysia for facilities provided. Last but not least, this study would not have been possible without the research grant by IPS Graduate Fund 1001/PJJAUH/834068 and USM Fellowship.

TABLE OF CONTENTS

CHAPTER 3 - MATERIALS AND METHODS

CHAPTER 4 - RESULTS

LIST OF FIGURES

LIST OF TABLES

LIST OF SYMBOLS AND ABBREVIATIONS

USM	Universiti Sains Malaysia
USMARC	Universiti Sains Malaysia Amphibian Reptile Collection
o ” ’	degree minute second
a.s.l.	above sea level
MW	mouth width
SVL	snout to vent length
df	degree of freedom
P	probability
VMAX	maximum prey volume
VMIN	minimum prey volume

PEMAKANAN LIMA SPECIES KATAK YANG BIASA DITEMUI DI KAWASAN TERGANGGU DI UTARA SEMENANJUNG MALAYSIA

ABSTRAK

Analisis kandungan perut lima spesies katak yang biasa dijumpai di kawasan terganggu , iaitu *Duttaphrynus melanostictus* (Schneider, 1799), *Polypedates leucomystax* (Gravenhorst, 1829), *Microhyla heymonsi* (Vogt, 1911), *Microhyla butleri* (Boulenger, 1900) dan *Microhyla fissipes* (Boulenger, 1884), telah dijalankan dari Oktober 2012 hingga Mac 2013. Sebanyak 387 spesimen telah dikumpulkan dari dua belas terganggu kawasan di utara Semenanjung Malaysia, yang terdiri daripada 120 ekor *D. melanostictus,* 86 ekor *M. butleri,* 77 ekor *M. fissipes,* 53 ekor *M. heymonsi* dan 51 ekor *P. leucomystax*. Sebanyak 42 kumpulan mangsa telah dikenal pasti (sekurang-kurangnya ke tahap famili), dengan 14 kumpulan mangsa yang juga ditemui dalam persekitaran kawasan kajian dengan menggunakan perangkap pelekat. Platygasteridae, Formicidae dan Araneae hadir dalam diet kesemua spesies katak. Makanan dominan untuk *D. melanostictus* ialah semut (F= 30.91) dan kumbang tanah (F=13.18), untuk tiga spesies katak Microhyla ialah semut (F>50), dan untuk *P. leucomystax* ialah rama-rama (F=18.52) dan kumbang daun (F=14.81). Formicidae merupakan mangsa utama yang ditemui kerana banyak terdapat dalam persekitaran (43.20%). Analisa pemakanan menunjukkan bahawa *P. leucomystax* ialah pemangsa umum atau "generalist" dan *D. melanostictus* adalah generalist yang memberi

keutamaan kepada semut. Sementara itu, *M. butleri* , *M. fissipes* , dan *M. heymonsi* ialah pemangsa pakar semut atau "ant-specialist". Kajian ini juga menunjukkan bahawa saiz mangsa *D. melanostictus* mempunyai korelasi positif dengan lebar mulut (PVmax<0.0001, PVmin=0.0017). Bagi katak Microhyla, lebar mulut tidak memberi kesan kepada saiz mangsa (P>0.5). Dari segi kepelbagaian mangsa, hanya *D. melanostictus* dan *P. leucomystax* menunjukkan kepelbagaian mangsa tinggi. Namun keluasan nic pemakanan kesemua spesies menunjukkan nilai yang rendah, dengan pengecualian kepada *P. leucomystax*. Pertindihan nic adalah sangat kecil antara katak-katak Microhyla dengan *P. Leucomystax*.. Kajian ini menyumbang sedikit pemahaman kepada evolusi myrmecophagy pada amfibia secara keseluruhannya.

DIET OF FIVE COMMON ANURANS FOUND IN DISTURBED AREAS IN NORTHERN PENINSULAR MALAYSIA

Abstract

Analyses of stomach contents of five species of commonly found disturbed area frogs, namely *Duttaphrynus melanostictus* (Schneider, 1799), *Polypedates leucomystax* (Gravenhorst, 1829), *Microhyla heymonsi* (Vogt, 1911), *Microhyla butleri* (Boulenger, 1900) and *Microhyla fissipes* (Boulenger, 1884), were carried out from October 2012 until March 2013. A total of 387 specimens were collected from twelve disturbed areas in northern Peninsular Malaysia, consisting of 120 individuals of *D. melanostictus*, 86 *M. butleri*, 77 *M. fissipes*, 53 *M. heymonsi* and 51 *P. leucomystax*. A total of 42 prey groups were identified (at least to family level), with 14 of the prey groups also found in the environment using glue traps. Platygasteridae, Formicidae and Araneae were present in the diets of all five anuran species. The dominant prey items for *D. melanostictus* were ants (F=30.91) and ground beetles (F=13.18), for the three microhylids were ants (F>50), and for *P. leucomystax w*ere moths (F=18.52) and leaf beetles (F=14.81). Formicidae was the major prey of five anurans, because of its abundance in the environment (43.20%). Diet analysis shows that *P. leucomystax* was most probably a generalist and *D. melanostictus* was a generalist with preference for ants. Meanwhile, *M. butleri*, *M. fissipes*, and *M. heymonsi* were ant-specialists. This study also shows that prey size of *D. melanostictus*

was positively correlated with mouth width (P_{Vmax}<0.0001, P_{Vmin}=0.0017). For the microhylids, mouth width had no effect on prey size (P>0.5). In terms of prey diversity, only *D. melanostictus* and *P. leucomystax* showed high prey diversity, but for dietary niche breadth, all species showed low values, except for *P. leucomystax*. The least niche overlap was shown between the microhylids and *P. leucomystax*. This study contributes some understanding to the evolution of myrmecophagy in amphibians as a whole.

CHAPTER 1

Introduction

1.1 Introduction

Malaysia is one of the hotspots for tropical biodiversity in the world (Ibrahim & Nurul Dalila, 2008). The warm and moist tropical climate of Malaysia encourages the growth and evolution of the animals in the region, especially the amphibians that thrive in such environment. Within its 329, 847 km^2 of land, Malaysia is currently home to 233 species of amphibians, with at least 63 endemic species found exclusively in the country, and more waiting to be discovered in its forest (IUCN, 2008). Just within the last five years, scientists have discovered 7 new species and 1 new genus in Peninsular Malaysia, while 11 species were discovered in Borneo. Yet, there are still many amphibians to be discovered, and also those look-alikes that were wrongly grouped as a single species pending to be re-described as new species. As of year 2009, Chan et al. (2010) listed as many as 107 species of amphibians in Peninsular Malaysia.

Frogs and toads are integral parts of the food web in the ecosystem. As tadpoles, they graze on a variety of algae and detritus gleaned from the water column or found on the bottom substrates. On the other hand, they become food for predators, such as insects and fish. As adults, frogs and toads eat insects and other small invertebrates and vertebrates, and, in turn, are preyed upon by snakes, birds, humans and a host of other predators (Ibrahim, 2004).

Anderson et al. (1999) mentioned that the knowledge of diet and feeding ecology is crucial to the understanding of life histories, population fluctuations, and

the impact of habitat modification on anuran populations. The diet composition of an anuran is influenced by prey size, mobility, availability, palatability and nutritional value, and also by the frog's morphology and behaviour, which are subjected to evolutionary processes, and the anuran's previous experience (Santos et al., 2003). Amphibians are carnivores, and, are opportunistic feeders that target moving prey crossing their line of vision, although the size of their chosen prey is limited by their gape width (Toft, 1981). They are known to prey on a wide spectrum of invertebrates including annelids, arachnids, centipedes, millipedes, molluscs and especially insects (Anderson et al., 1999; Dietl et al., 2009; Hirai & Matsui, 2000a, 2000b, 2000c, 2002; Ibrahim & Nurul Dalila, 2008; Santos et al., 2004; Solé et al., 2009).

Considering the vast variety of anurans available in Malaysia, there are relatively few publications on the diet of anurans (Berry & Bullock, 1962; Berry, 1965, 1966, 1970; Elliott & Karunakaran, 1974; Erftemeijer & Boeadi, 1991; Ibrahim & Nurul Dalila, 2008; Ibrahim & Sofrina, 2001; Ibrahim, 2004; Kueh et al., 2010; Yap & Ibrahim, 2012), as most of the studies focused on refining the inventory (Berry, 1975; Chan et al., 2010; J. L. Grismer et al., 2004; Grismer et al., 2006; Grismer et al., 2010; Ibrahim at al., 2008; Inger & Tan, 1996; Norhayati et al., 2005; Shahriza et al., 2011; Shahriza, Ibrahim, & Shahrul Anuar, 2011), breeding (Berry, 1964; Church, 1960; Inger & Bacon, 1968; Inger & Greenberg, 1963), ecology (Emerson & Inger, 1992; Inger, 1969, 2003), descriptive notes and zoogeography (Berry, 1975; Boulenger, 1912; Das, 2008; Inger & Iskandar, 2005; Inger et al., 2009; Inger & Stuebing, 1992, 2005; Inger & Voris, 2001) of the amphibians.

Common Asian toad (*Duttaphrynus melanostictus*), Painted chorus frog (*Microhyla butleri*), Dark-sided chorus frog (*Microhyla heymonsi*), Paddy rice frog (*Hylarana erythraea*), and Golden tree frog (*Polypedates* cf. *leucomystax*) are the five

species commonly found living commensally with human, and can be found in many disturbed areas in Malaysia. There is however a lack of studies on these species, especially on their diet. As Breen (1974) mentioned, mankind has only thorough studied of a handful of very common species, but of the vast majority we know little. Our appreciation of nature is heightened when our awareness of the environment is increased, and frogs are well-known bio-indicators of the environment. For this reason, the aims for this study were to determine the relative abundance of these species in a disturbed area and their diets in order to gain more insights on the animals.

1.2 Objectives

1. To determine the prey items consumed by the five anurans commonly found in disturbed areas (*Duttaphrynus melanostictus*, *Microhyla butleri*, *Microhyla fissipes*, *Microhyla heymonsi*, and *Polypedates leucomystax*) in order to infer predation strategies.
2. To determine the correlation between the mouth width of the anurans and volume of the prey items in order to infer predation strategy of each species.
3. To study the difference in morphometry and prey compositions of male and female anurans.
4. To determine the niche breath and niche overlap of these anurans in order to infer whether there is a strong or weak interspecific competition between the five species.

CHAPTER 2

Literature Review

Herpetology, is the study of two distinct clades of vertebrata, the reptiles and amphibians, known to laymen as the "creepy crawlies". The term is derived from ancient Greek "herpeton", which carries the meaning "crawling things". Herpetology examines the natural history, physiology, and phylogenetic of these animals and their ecological importance at a global scale (Porter, 1972). The study on amphibian alone is a sub-discipline of herpetology that is termed batrachology.

2.1 Amphibians in General

“Amphibia”, derived from an ancient Greek word "amphibios" meaning both kinds of life, was first coined by Linnaeus in year 1758 for an assemblage of half terrestrial and half aquatic vertebrates with amphibious habit. Adaptive radiation in modern amphibians is chiefly reproductive and coping with the need of water for breeding (Webb et al., 1981). Amphibians are animals that live alternately on land and in water. These animals in general have a soft, moist scaleless skin; their eggs lack a protective shell; and they typically develop through an aquatic larval stage followed by metamorphosis transformation into an adult that may be amphibious or wholly aquatic. Amphibians prefer moist, humid habitat and constantly bathe in water to maintain their moist skins and to avoid water loss (Duellman & Trueb, 1986).

Amphibia, in general, is defined as an ectothermal vertebrate with a smooth or rough glandular skin, no scale or if scales are present, they are hidden in the skin

(Noble, 1954). In general, taxonomist tried to classify and categorize the amphibians according to shared features and structures, which are then further supported by the behavioural types and evolutional relationships of the amphibians. Thus, the classification and taxa of the amphibians helps predict the behaviour and life cycle of unfamiliar amphibians by comparing with others under the same taxon. The class Amphibia is divided into 3 surviving orders: Anura, Caudata, and Gymnophiona. Caecilians are classified under the order Gymnophiona, salamanders and newts are under the order Caudata, whereas frogs and toads are from order Anura.

Caudata has an elongated body, a long tail, and with exception of Sirenidae family, two pairs of limbs that are of the same size. Like all amphibians, caudates are restricted to wet places. Among Caudata, there are species that gave up their amphibious lifestyle. They are either totally aquatic or highly terrestrial in their adult stage. Aquatic caudates are commonly called newts, and live in streams, lakes, marshes, and even in subterranean waters. Terrestrial caudates, the salamanders, may be found burrowing under earth, hiding between stones, holing up in caves or in trees. For example, *Dendrotriton* sp., a genus of plethodontid salamanders that is also called bromeliad salamanders, live and reproduce in pineapple plants (family, Bromeliaceae) (Wake, 1987). There is a unique phenomenon among caudates, where the amphibian reaches sexual maturity in its aquatic larval stage. This phenomenon is known as paedogenesis, and can be seen in the axolotl (*Ambystoma mexicanum*) (Webb et al., 1981).

Gymnophiona, or previously known as the order Apoda, are an obscure order of legless amphibians, known as caecilians. Caecilians can be found in tropical forests, burrowed in soft damp earth. Morphologically, caecilians resemble large earthworms, with cylindrical body lined with minute dermal scales, no limbs, and no tail (Webb et

al., 1981). Caecilians also have a unique tentacle beneath its functionless eye, presumably for sensory to compensate the loss of sight.

The order Anura, with the word Anura derived from ancient Greek meaning "without tail", is a diverse group of carnivorous frogs and toads. In a narrow sense, members of the family Bufonidae are considered as toads (Duellman & Trueb, 1986). Different species of anurans adapt to living in different habitat via intrinsic changes rather than extrinsic, by altering their physiology, behaviour, and reproductive methods (Webb et al., 1981). Anurans are ectothermic, tetrapod vertebrates with a generally smooth, moist and glandular skin (Noble, 1954). The skin of anuran is very permeable, thus they are often semi-aquatic and inhabit humid areas. Anurans are most conspicuous by the males' advertisement calls, which can be heard during the night or some times, even during the day.

2.2 Habitat and Distribution

Amphibians can be found all over the world, with the exception of Antarctica where the weather is extremely cold (Noble, 1954). They inhabit any type of habitat as long as there is enough moisture readily available. Globally, the distribution of anurans is more concentrated near the equator, thus there are higher species diversity and more endemic species in the tropical regions (Inger, 2005).

The classification of anurans is affected in some degree by the habitat of the anurans. For example, all anurans from the family Megophryidae are known leaf litter frogs, while those under family Rhacophoridae are all tree frogs. Due to its permeable skin and a life cycle that requires water to breed, anurans are bounded to areas with presence of water. However, anurans are fresh water species. Anurans cannot survive in salt water due to their inability to concentrate urine. Only a few species are able to tolerate the salinity of brackish water, such as *Fejervarya cancrivora* that are found living in mangrove areas of Malaysia (Inger & Stuebing, 2005).

Blessed with frequent rain and steady tropical climate, anurans are omnipresent in Malaysia. The majority of the anurans are found in and around the forest area, where humidity is high and the climate change is modest. Though nowadays, due to the expansion of human activities into the habitat of anurans, more and more anurans are becoming human commensal (Norhayati et al., 2005). The amphibian habitats that are in frequent contacts with human over a long period (disturbed areas) show fewer species and those where human presences are lesser or none (undisturbed areas) show higher number of species richness (Mohamed Ali Abdu Assalam, 2000). Though for the fewer species that live in man-altered environment, they thrive in numbers. The relationship between the diversity and abundance of amphibians, and the density of human population is complex (Porter, 1972).

2.3 Anurans in General

Anurans undergo metamorphosis and the life cycle comprises four main stages: egg, tadpole, froglet and adult. Anurans' egg is shell-less, the embryo is protected in highly permeable mucoid capsule, and thus must develop in moist situations (Duellman & Trueb, 1986). Eggs hatch into tailed larvae called tadpoles that grow quickly, and then metamorphose into small froglets. As the froglets grow, the tail shrinks until it's absent in the adult. There is no distinct neck in the froglet and adult stages, as the head of an anuran is externally continuous with the body.

An egg consists of the vitellus (the embryo) encompassed by several gelatinous layers. The number of eggs laid in a single brood differs from species to species. An amphibian may lays its eggs in forms of single egg that sink to the bottom of the breeding pond or are attached to submerged vegetation, in small packet of eggs, in strings or files wrapped around emergent vegetation (e.g. *Duttaphrynus melanostictus*), in a surface film that floats on the water surface (e.g. *Kaloula pulchra*), in clusters that are usually attached to emergent aquatic vegetation, or in a foam nest attached to overhanging vegetation (e.g. *Rhacophorus nigropalmatus*) (Duellman & Trueb, 1986; Haas et al., 2013; Porter, 1972).

A tadpole has a body and a tail. Its keratinous beaks and denticles serve as larval mouthparts, which differentiate it from a typical fish. There is also a single median spiracle in the larva that is characteristic of Orton's Type 3 tadpole. The tail of a tadpole consists of the centre axis of muscle segments, and crests that are the upper and lower fins (Duellman & Trueb, 1986).

Adult anurans have elongated tarsal bones, and complex pectoral girdle. There is an absence of a prefrontal bone. They have 5 to 9 presacral vertebrae, so that the trunk is shortened for jumps. Hind limbs are considerably larger and more muscular than fore limbs, as they are used for jumping and swimming. The radius and ulna of the arm are fused, and same goes with tibia and fibula of the leg. Their ankle bones, tibiale and fibulare, are both elongated. Most anurans are oviparous, some species are ovoviviparous and others viviparous, but all fertilisations happen externally (Md Hanapi & Ibrahim, 1986; Webb et al., 1981).

Matured amphibians show secondary sexual characteristics. In general, the more active males are inclined to darker dorsal colours compared to the females. Males also tend to be more self-coloured than the females, which may be more spotted, such as the brown tree toad (*Pedostibes hosii*) (Haas et al., 2013). On the other hand, females tend to be larger in size, with the exception of few species. Other examples of sexual dimorphisms include the pronounced differences in throat colouration in bufonids (whereby males have marked yellow colouration and females have none), enlarged tympana in males, enlarged thumb or presence of pad in males, skin or body projections in males, and presence of throat sac in males (Katsikaros & Shine, 1997; Webb et al., 1981).

Tropical anurans have acyclic breeding patterns and many species breed all year round, as studies found no evidence of seasonal trends in the reproductive activities of several rainforest anurans (Berry, 1964; Inger & Bacon, 1968; Inger & Greenberg, 1963; Inger & Voris, 1993). This is attributed to the constant climate of tropics. However, the anurans breeding periodicity is affected less by the variation in precipitation and more on the presence of water in their local environment (Alcala, 1962; Ibrahim, 2004; Inger & Voris, 1993). As their breeding rhythms are direct

adaptations to local extrinsic factors, rainfall variation may cause immediate shift in reproductive pattern, i.e. *Duttaphrynus melanostictus*'s peak spawning occurs with the influx of rainwater after an extended period of dry weather (Church, 1960).

Gross & Shine (1981) and Crump (1996) recognised six modes of amphibian parental care: egg attendance, egg transport, tadpole attendance, tadpole transport, tadpole feeding, and internal gestation in the oviduct. Parental care in amphibians increases survivorship of the offspring, and is most commonly found in geographical areas of correspondingly high species richness. Famous examples of amphibians that practice parental care include mid-wife toad (*Alytes obstetricans*), Surinam toad (*Pipa pipa*), Darwin's frog (*Rhinoderma darwinii*) and more.

Generally, anurans do not possess unique features, and each species is defined by a combination of characteristics. As of current, Frost (2013) listed as many as 7044 species of amphibians known worldwide. These are 6200 species of Anura (88%), 652 species of Caudata (9%) and 192 species of Gymnophiona (3%). IUCN, Conservation International, & NatureServe (2008) assessment (also known as IUCN Red List) of 6260 species of amphibian determined that as many as 2030 species or nearly one-third of the species (32.4%) is globally threatened or extinct. About 1533 species (24.5%) cannot be assessed due to the lack of data, though among these species may contain a significant proportion of threatened species. IUCN (2008) also assesses that the threat level for Anura was average, 31.6% (1749 species) currently threatened or extinct. On the other hand, about half of the Caudata species (49.8%) are threatened or extinct. The seemingly low threat of Gymnophiona (3.4%) however is due to the majority of the species do not have sufficient data for assessment. Family-wise, Sooglossidae Noble, 1931 and Calyptocephalellidae Reig, 1960 are most vulnerable, as all of the species under both families are either vulnerable or endangered.

Malaysia, with its west peninsular joined to the Southeast Asia mainland, and Sabah and Sarawak from Borneo Island, is home to 8 genera and 233 species of amphibians (Chan et al., 2010; Haas et al., 2013; Norhayati et al., 2009). Over 90% of them are anurans. In Peninsular Malaysia itself, Chan et al. (2010) listed a total of 105 anuran species from six families, consisting of 18 species from Dicroglossidae, 10 species from Megophryidae, 18 species from Bufonidae, 20 species from Microhylidae, 18 species from Ranidae and 21 species from Rhacophoridae.

Dicroglossidae Anderson, 1871 has previously been grouped together as Ranidae until recent molecular works suggested otherwise. This family is distributed in Europe, Northwest Africa and throughout Asia. There are only 181 species of frogs from this ancient clade (Frost, 2013). There are very few external features that unite the species within this family, though they generally resemble the true frog of ranids. All species of this family are ground-dwelling and are usually found close to bodies of water. Malaysia is currently home to 4 genera and 25 species of dicroglossids (Chan et al., 2010; Haas et al., 2013). Malaysia's dicroglossid includes two of the country's largest frog species, peat swamp frog (*Limnonectes malesianus*) and Blyth's river frog (*Limnonectes blythii*).

The Asian toad of Megophryidae Bonaparte, 1850 are a family of South Asian frogs spread from Pakistan and India to the Southeast Asian countries such as Malaysia, Philippines and Indonesia. Frost (2013) listed 10 genera and 171 species from this family. They are the most ecologically and morphologically diverse group of non-neobatrachian frogs. In Malaysia, Megophryidae family consists of 6 genera and 29 species. Megophryids are small to medium sized frogs, with the snout-vent length ranging from 20 mm to 125 mm (Norhayati et al., 2005). They are nocturnal species that burrow in the day (Breen, 1974). Most Megophryids are also poor jumpers, and

prefer to crawl along, even when disturbed. Another common feature of this family is the possession of a flat tongue and a spade-like hind foot, which gives rise to the name "spadefoot toad" (Breen, 1974).

The colouration of Megophryids is typically dull and earth tone. Certain species of Megophryids possess skin projections that resembled dead leaves, for example, horned frog (*Megophrys nasuta*) has dermal projections all over its body ridges, so that when among the forest litter, it can easily mimicked one of the fallen leaves (Duellman & Trueb, 1986). Megophryids in Malaysia are experts in camouflaging themselves among ground litters, which earns them the name "leaf-litter frogs" (Berry, 1975).

Bufonidae Gray, 1825 or the true toads made up the third largest family of the anurans with 571 species scattered all over the world (Frost, 2013). Presently, approximately 44.1% of the total species in this family are considered to be either threatened or extinct and 6 species has already lost forever to mankind (IUCN, 2008). Being the second largest family in Malaysia, Bufonids face the same grim situation when 58% of the total 42 species are threatened (Norhayati et al., 2005). The six genera of Malaysian Bufonids made up of the genus *Ansonia*, with 19 species, followed by *Pelophryne* (8 species), *Ingerophrynus* (5 species), *Pedostibes* (3 species), *Phrynoidis* (2 species), and one species each under these genera: *Bombina*, *Duttaphrynus*, *Leptophryne*, *Pseudobufo* and *Sabahphrynus*.

Bufonids are found throughout temperate and tropical regions, with the exception of Australopapua region, Madagascar and Oceania. The only toad found east of the Wallace's Line is cane toad (*Rhinella marina*, previously known as *Bufo marinus*) that is introduced in Australia along with the sugar cane from South America

(Frost, 2013). All bufonids are very much alike in over-all appearance. Compared to frogs, toads are typically dull in colouration, have dry warty skins, and lack true teeth (Breen, 1974).

Microhylidae Günther, 1858, as the name indicates, comprises of a family of small-size frogs, though some among them reach medium-size. The snout-vent length of these microhylids range from 7 mm to 100 mm. Nevertheless, the most distinguishable feature that defines this family is their narrow mouths. Therefore, they are collectively known as narrow-mouth frogs (Frost, 2013; Norhayati et al., 2005). Microhylids have rounded bodies, and some species namely the bullfrogs will further puff up their size when under threat, as seen on *Kaloula pulchra*. Despite their relatively small size, microhylids make the loudest calls that preceded their presence (Haas et al., 2013; Norhayati et al., 2005).

Since this is a family of tiny frog, it is no wonder that the world's smallest vertebrate is categorized under its wing. Formally described at year 2012, *Paedophryne amanuensis* from New Guinea is the smallest amphibian measured at 7 mm long. The tiny species of *Paedophryne* genus from the eastern forests of Papua New Guinea were identified quite recently, as they are hard to spot, camouflaging among forest floor leaf-litters, and have calls that resembled insects (Black, 2012).

A relatively young clade of anuran family in the term of phylogenetic evolution, Microhylidae has over 519 species distributed worldwide, with the exception of the Antarctic continent (Frost, 2013). Malaysia is home to 40 species of microhylids under 9 different genera. Microhylids are separated into burrowing group and arboreal group. Burrowing microhylids usually emerge after heavy rains, usually to breed (Norhayati

et al., 2005). Arboreal microhylids take shelter in tree holes, or hiding underneath leaves of epiphytes.

Frogs from the family of Ranidae Rafinesque, 1814 are considered the "true frogs". The layman generalization of the forms and functions of frog comes from ranids. Ranidae is the world fifth largest anuran's family comprising 355 species (Frost, 2013). Ranids are Old-World frogs, its distribution world-wide with the exception of temperate South America, West Indies, most of Australia, and Oceanic islands. In Australia only a single species, *Hylarana daemeli*, is recorded from northern Queensland and north-east Northern Territory of Australia. Ranidae in Malaysia consisting of 9 genera; 38 species (Chan et al., 2010; Haas et al., 2013). Ranid's pectoral girdle is firmisternal, with the sternum fused to the pectoral arch and the epicoracoidal cartilages fused (Duellman & Trueb, 1986). Appearance-wise, its skin is smooth and moist, there is a dorso-lateral skin fold from behind the eye to the hind limb and maxillary teeth are present. Ranids are riparian and cosmopolitan, found in in forests, swamps, paddy fields, lakes, rivers, and even in garden ponds.

Ranids can be as small as 20 mm to 300 mm in snout-vent length. The largest frog in the world – goliath frog (*Conraua goliath*) endemic to Cameroon and Equitorial Guinea, comes from the family Ranidae (Amiet, 2004). This family also housed Malaysia's largest frog – Blyth's giant frog (*Limnonectes blythii*), which is also an economically important species in the country. Tanah Rata wart frog (*Limnonectes nitidus*) is the only endangered ranids in Malaysia (Leong & Yaakob, 2004).

If the ranids are "true frogs", Rhacophoridae Hoffman, 1932 are a family of Old World tree frogs that occurs in Asia and Africa. Two subfamilies are recognized under Rhacophoridae: Buergeriinae, and Rhacophorinae (Frost, 2013). Due to the

arboreal nature of the frogs in this family, they are commonly known as shrub frogs, bush frogs and tree frogs, and most spectacularly, the gliding frogs in this family are also called flying frogs. In Malaysia, Rhacophoridae is the largest family comprising 52 species, and 6 genera.

Rhacophorids' size range from 15 mm to 120 mm. Morphologically, tree frogs are fully adapted to living in the canopy. They seldom leave the protection of the foliage unless to mate and lay eggs at the nearest streams. As such, rhacophorids have enlarged toe disks to facilitate climbing and to hold onto tree branches and leaves; full webbings between the fore fingers and hind toes for gliding through the air while they leap from one tree to another. They also possess flattened bodies, no ribs, broad flat skulls, dentate upper jaw, and cartilaginous intercalary elements between the terminal and penultimate phalanges (Norhayati et al., 2009).

2.4 Classification of Frog and Toad

While there are no true clear guide to the classification of frogs and toads, there are a few external features or characteristics that are used to distinguish between a frog and a toad (Fig. 2.1). In a broad sense, toads are those anurans from the family Bufonidae, also called bufonids.

In general, toads have dry, warty skin and possess elevated ridges (cranial crests) between and at the back of the eyes. In addition, a prominent, raised, glandular area (parotoid gland) behind the eye is found on toads (Duellman & Trueb, 1986). Toad tends to have rounder, blunter snout compared to frog. Since toads generally

crawl, and only make short distance jump, they have stout but shorter hind legs. With the exception of some tree toads, toads commonly have pointy fingers with no toe disks. Webbings between the toes are usually absent in toads.

On the contrary, true frogs have moist, smooth skin with or without a ridge (dorsolateral fold) extending from the ear drum (tympanum) along the side of the back to the hip or groin area (Berry, 1975). Most frogs have longer pointy snout. They also have long slim but muscular hind limbs for long leaps. Almost all frogs have toe disks on their hind legs, some more prominent than others, though toe disks may or may not be absent on the fore fingers (Duellman & Trueb, 1986). Due to their liking of wet habitat, frogs are likely to have webbings between their toes to help them wade through water.

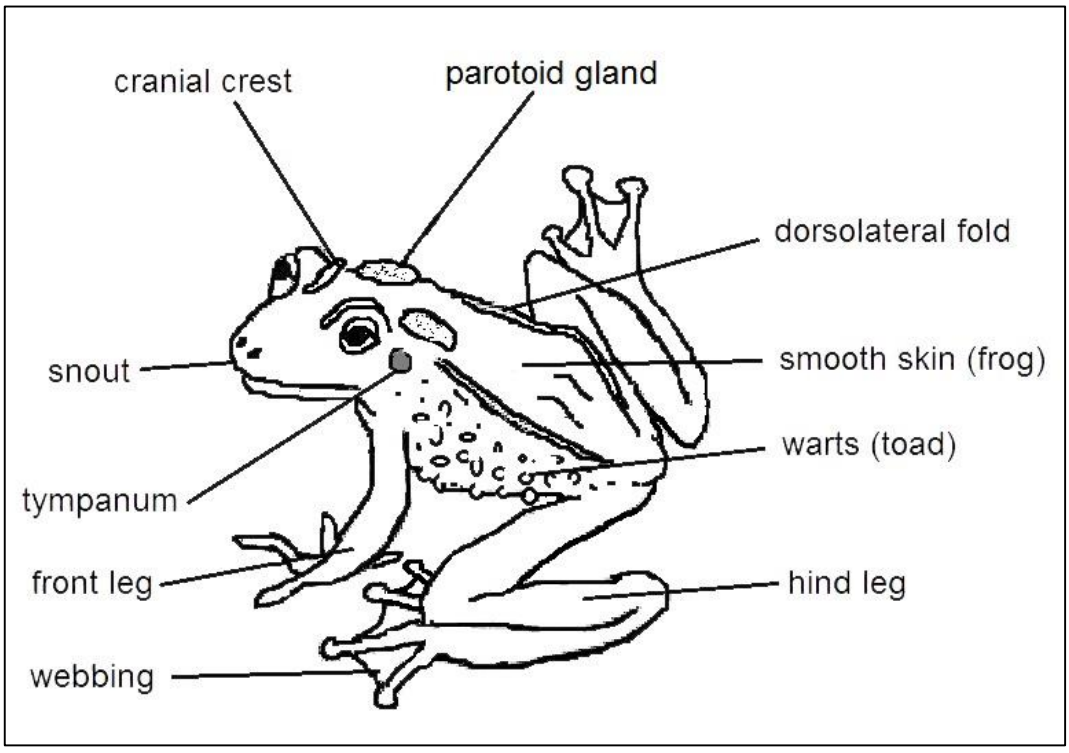

Figure 2.1 External characteristics of frogs and toads.

2.5 Descriptions of Specimens

2.5.1 *Duttaphrynus melanostictus* Schneider, 1799

Classified under family Bufonidae, *D. melanostictus* used to be named *Bufo melanostictus*, *Bufo tienhoensis*, and *Ansonia kamblei* (Frost, 2013). It is commonly called common Sunda toad, Asian common toad, Asian toad, Javanese toad, or black-spectacled toad (Van Dijk, Iskandar, Lau, et al., 2004). Local calls it katak puru biasa, or simply katak puru. The average snout-to-vent length (SVL) of an adult male ranges from 57 mm to 83 mm, and female ranges from 65 mm to 85 mm (Norhayati et al., 2009).

As with most toads, this species has a stocky body, with dark elevated supraorbital and supratympanic bony ridges on its head, but no parietal ridges (Norhayati et al., 2005). *Duttaphrynus melanostictus* can be easily differentiated from other toads with its distinctly large and elongated oval-shaped parotoid glands on the back of its neck (Fig. 3.3). The parotoid glands are usually covered with black spots. Its head width is longer than head length. Its snout is obtusely pointed. It has distinct tympanum, which is about half the size of its eye. The finger and toe tips are blunt. There is presence of supernumerary metacarpal tubercles on fore limb and two metatarsal tubercles on hind limbs, and also conspicuous subarticular tubercles on its limbs. Hind limbs are more than half webbed between the toes, with third and fifth toes usually with one phalanx free of web, and fourth toe with three phalanges free of web (Berry, 1975). The dorsal skin is rough and overall very warty. Its colour varies from greyish to reddish-brown or yellowish. The ventral is usually pale brown or pale yellow in colour. Male *D. melanostictus* has distinct yellow-tinted chin.

This species spread widely from Bangladesh, Southern China to South East Asia. *Duttaphrynus melanostictus* is ubiquitous in disturbed areas all over Malaysia. It is a well-known human commensal species, found in disturbed open areas, villages, towns and rarely in primary forest. *Duttaphrynus melanostictus* is mainly a lowland disturbed areas species, hard to be found in a closed forest. Hence, it's a good indicator of habitat disturbance. It has also been recorded from sea level up to 1,800 m a.s.l. Listed as least concern (LC) in the IUCN Red List of Threatened Species, this species global population is speculated to be increasing (Van Dijk et al., 2004).

Typically found hiding under ground cover or hiding under low foliage, adults *D. melanostictus* are terrestrial and only gather at nearby freshwater for breeding. This species breeds in still water bodies, such as permanent or temporary pond and pool, or slow-moving rivers. The eggs are laid in strings that are strewn across the bottom of the water or the aquatic vegetation under water. In a tropical country, such as Malaysia, *D. melanostictus* breeds all year round as long as there is ample standing water nearby (Berry, 1964).

2.5.2 *Microhyla butleri* Boulenger, 1900

Commonly called Butler's rice frog, painted chorus frog, tubercled pygmy frog, or katak padi Butler in local tongue, it is a tiny frog under the family Microhylidae. Male's SVL ranges from 21 mm to 23 mm, whereas female's SVL ranges from 23 mm to 26 mm (Norhayati et al., 2009).

It has a rounded snout, and no visible tympanum. Its finger tips are dilated into small but well-developed disks, with its first finger much shorter than the second finger. The tips of its toes are like those of fingers, disks with circum-marginal groove and median notch, and webbed at the base. The hind limbs subarticular tubercles are small. There are two very small metatarsal tubercles (Berry, 1975).

Its skin maybe smooth or some time speckled with smooth warts. The dorsal is greyish with symmetrical dark brown, wavy markings which extended down onto its sides, forming bars on the hind limbs (Fig. 3.3). Markings sometimes accompanied by oblique white streak. The insertions are speckled with several granules or glandular tubercles (Norhayati et al., 2005). Its sides and limbs is pale reddish in colour, with or without small scarlet spots on the sides. Occasionally, specimens are found with white dot on the end of the snout. The ventral is whitish, with throat and breast speckled with brown (Berry, 1975).

This is known to be found in China, Hong Kong, Taiwan, Vietnam, Myanmar, Thailand, Malaysia and Singapore. This species inhabits lowland forests, freshwater wetlands, cultivated areas and grasslands where they are usually found on the ground among low vegetation, such as bushes, shrubs and grasses. It is found up to 1,000 m a.s.l. (Norhayati et al., 2005). *Microhyla butleri* has a very loud and distinct call that this species' presence is always preceded by the choruses of their calls.

While this species is abundant in Southeast Asian countries, its distribution is deemed rare in Taiwan and fragmented in China. It is considered a Class II protected species in Taiwan. Contradictory to its ever presence in local paddy fields, no population of *M. butleri* was ever found in the paddy field in Taiwan, presumably due to the use of harming chemical pesticides and fertilizers. Nonetheless, it is still listed

as least concern (LC) in the IUCN Red List of Threatened Species justified by the species wide distribution, its ability to tolerate a broad range of habitat, and the unlikelihood of the presumably large population to decline in the near future (Van Dijk et al., 2009).

M. butleri is a terrestrial riparian species. Due to its small size and the inability to hold a large amount of water in its body, *M. butleri* does not stray far from moisture. This species breeds in relatively permanent standing water, for example, ponds, pools, marshes, and paddy field. It has no reproductive cycle in relation to the climate (Berry, 1964).

2.5.3 *Microhyla fissipes* Boulenger, 1884

Also named *Microhyla eremita*, this is another small frog under family Microhylidae (Frost, 2013). This species does not actually have its own common name due to the fact that it is easily mistaken as ornate narrow-mouthed frog (*Microhyla ornata*) from South Asian region. SVL of males range from 22 mm to 27 mm, while females range from 25 mm to 28 mm.

Morphologically, this species is similar to *M. butleri*, with little difference. Appearance-wise, *M. fissipes* is indistinguishable from *M. ornata*. It has smooth skin. Its dorsal back is reddish or greyish olive coloured, with a large dark brown marking on the centre, beginning between the eyes and widening as it extends to the hind part of the body. On each side of this marking, wavy dusky lines are often present (Fig. 3.3). There is a dark band along the side of the head and body. Its hind limbs maybe

with or without dark cross-bars. The ventral throat and chest are generally greyish brown, dotted with white, whereas the remainder of the lower ventral surface whitish, and unspotted (Norhayati et al., 2009).

Prior to the study by Matsui et al. (2005), there is confusion between *M. fissipes* and *M. ornata*, resulted in many scientists wrongly identified *M. fissipes* in the Southeast Asia region as *M. ornata*. However, the boundary between these two species is still not clear, and it has been arbitrarily set at the border of Myanmar until there is further clarification on the matter.

Known to be omnipresent all over mainland Southeast Asia, with only a record from a single locality in the north of Sumatra, Indonesia, this species inhabits lowland forests, disturbed areas where they are usually found on the ground or low vegetation, and around paddy fields. Sub-fossorial in habit, it can also be spotted hiding among forest floor leaf-litter. There is report that *M. fissipes* was found up to 2,000 m a.s.l. Like *M. butleri*, *M. fissipes* is considered least concern (LC) by IUCN Red List of Threatened Species in view of its expanded distributions, the sheer number of populations, its high adaptation to various habitats, and the improbability of sudden decline (Lau et al., 2008).

M. fissipes is a species nocturnal in nature, only active diurnally during rainy season for breeding (Lau et al., 2008). While there is no known study on *M. fissipes* breeding rhythm, it is believed that it has a noncyclic pattern similar to its closest cousin *Microhyla heymonsi* and *M. butleri*. It lays clutches of egg mass at the surface of both permanent and temporary still water bodies.

2.5.4 *Microhyla heymonsi* Vogt, 1911

Another small frog under family Microhylidae, it is commonly called dark-sided chorus frog, Taiwan rice frog, arcuate-spotted pygmy frog, or locally, katak bising. SVL of adult males range from 16 mm to 21 mm, while SVL of adult females range from 22 mm to 26 mm (Berry, 1975).

It has a slight glandular fold from posterior corner of the eye to its fore limb. Morphologically similar to *M. butleri*, *M. heymonsi* is the easiest to identify microhylid. It has smooth dorsal and ventral skins. Dorsally pinkish or greyish in colour with a black lateral band extending from the snout tip to the vent, and entirely covering the sides of the head (Fig. 3.3). This band is sharply defined above but merging gradually into the colour of the bellow beneath. A fine white vertebral line form from its snout to vent. There is a small characteristic black mark on each side of the line on the middle of the back. There may be an additional pair of similar but smaller marks between its shoulders. Its ventral skin colour is dirty white (Berry, 1975).

A widespread species found in China and most of South East Asia. This cosmopolitan species can usually be found alongside *M. fissipes* as they share the same microhabitat. *Microhyla heymonsi* inhabits mainly disturbed areas, such as riverbanks, gardens, paddy fields, grasslands, savannah forest and any patch of secondary vegetation. It is known to live up until an altitude of 945m a.s.l. (Imbun et al., 2010). Due to its widespread distribution, large global population, high tolerance to different habitats, and unlikely drop in numbers, IUCN Red List of Threatened Species places *M. heymonsi* as least concern (LC). Nevertheless, it is a Class II protected species in Taiwan (Van Dijk et al., 2004).

One of the noisiest frogs around (hence the local name “katak bising”, which can be literally translated to “noisy frog”), this frog’s loud calls can be heard all year round. This species reproductive pattern is acyclic and independent of the climate (Berry, 1964). It breeds in temporary rain puddles, ditches, marches, paddy fields, small streams and also slow-flowing rivers.

2.5.5 *Polypedates leucomystax* Gravenhorst, 1829

Classified under family Rhacophoridae, its vernacular names include four-lined tree frog (as some individuals have four longitudinal dorsal stripes, Fig. 3.3), common tree frog, Java whipping frog, brown tree frog, Malayan house frog, Malayan tree frog, white-lipped tree frog, bamboo tree frog, house tree frog, jar tree frog, and stripe tree frog. This species is considered a species complex because the exact demarcation between close species is still cryptic. Therefore, myriad nomenclature actually point to this species, i.e. *Hyla leucomystax, Hyla sexvirgata, Hyla quadrilineata, Hyla leucopogon, Hyla quadrivirgata, Polypedates rugosus, Polypedates quadrilineatus, Limnodytes celebensis, Rhacophorus maculatus var. quadrilineata, Hylorana longipes, Rhacophorus leucomystax, Rhacophorus leucomystax leucomystax, Rhacophorus leucomystax quadrilineatus, Rhacophorus leucomystax sexvirgata, Rhacophorus leucomystax quadrilineata, Rhacophorus maculatus leucomystax, Rhacophorus maculatus himalayensis, Hyla wirzi, Rhacophorus (Polypedates) leucomystax, Rhacophorus (Polypedates) quadrilineatus, Rhacophorus kampeni, Rhacophorus (Rhacophorus) kampeni, Rhacophorus (Rhacophorus) leucomystax, Rhacophorus (Rhacophorus) himalayanus, Rhacophorus*

(Rhacophorus) leucomystax leucomystax, Rhacophorus (Rhacophorus) wirzi, and *Rhacophorus leucomystax quadrilineatus* (Frost, 2013).

P. leucomystax can grow up to 80 mm in snout-vent length. Its head length is longer than its head width. With a pointed round snout, it has distinct round tympanum that is about ¾ of its eye diameter. The tips of its fingers dilated into disks with circum-marginal grooves, with the widest disks about $^{2}/_{3}$ of its tympanum diameter. These grooves provide suction at the finger tips, which in turn giving *P. leucomystax* the ability to climb on any vertical surface (Inger & Stuebing, 2005). The webbings between its first two fingers do not reach the subarticular tubercles, and less developed in its outer fingers. Its toe tip disks is smaller than those of its fingers, but the webbings usually reach the bases of disks on outer edge of first three toes and on inner edge of fifth toe, and the fourth toe has two phalanges free of broad web on outer edge (Berry, 1975).

Its skin smooth dorsally and ventrally, with the exception of adult male that has coarse granules on its throat. A broad, smooth-edged flap of skins is formed along the forearm and tarsus expanding along outer edges of its fourth finger and fifth toe. There is a broad round flap at the heel. Supratympanic fold is also present. *Polypedates leucomystax* dorsal colour is highly variable, from brown, light tan or grey, with or without dark interorbital bar, dark cruciform mark or dark, dorsal spots. Its dorsum is usually spotted irregularly with brown, black, blue, yellow or orange. There are dark cross-bars on its hind limbs. The ventral surface is usually whitish or pale yellowish in (Norhayati et al., 2009).

Though its geographical distribution is still provisional, there were records of *P. leucomystax* found in Nepal, China, India and throughout Southeast Asia. So far, it